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Double-stranded GFP RNA was prepared as described in Example I. Rat cells were transfected with pEGFP-N1 and double stranded GFP RNA using a standard transfection procedure. First, cells (~2x 10^8 per well) were seeded in a six-well tissue culture plate in 2 ml of DMEM with 10% FBS. The cells were then incubated at 37°C in a CO_2 incubator until they were about 70-80 % confluent (i.e., 18-24 hours)..

Pursuant to 37 C.F.R. §1.121, these paragraphs are also shown in Appendix A with notations to indicate the changes made.

In the Figures

Please replace Figure 13 with the enclosed substitute Figure 13.

In the Claims

Please cancel claims 8, 11-14, 33-38, 40, 41, 49-56, and 58-60, without prejudice.

Please amend claims 1, 4-6, 9, 10, 15-17, 25, 31, 32, 39, 42-48, 57, and 61 as indicated below, and add new claims 62-74. The new and amended claims are provided below in clean form. Pursuant to 37 C.F.R. §1.121, new and amended claims are also shown in Appendix A with notations to indicate changes made (for convenience, all pending claims, including those added hereby, are provided in Appendix A).

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1. (Amended) A method for attenuating the expression of a target gene in a vertebrate cell comprising supplying the cell with a double stranded RNA in an amount sufficient to specifically attenuate expression of the target gene, wherein one of the strands of the double stranded RNA is capable of hybridizing to the target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C, and provided that, when the double stranded RNA is supplied to the cell by delivery to the cell of double stranded RNA, the double stranded RNA is formed from single-stranded RNA that is purified in the absence of phenol or chloroform.

4. (Amended) The method of claim 1 wherein the target gene is a chromosomal gene.

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5. (Amended) The method of claim 1 wherein the target gene is an extrachromosomal gene.

6. (Amended) The method of claim 1 wherein the target gene is from a pathogen capable of infecting the cell.

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9. (Amended) The method of claim 1 wherein the vertebrate cell is a fish cell.

10. (Amended) The method of claim 1 wherein the vertebrate cell is a mammalian cell.

15. (Amended) The method of claim 1 wherein the double stranded RNA comprises a nucleotide sequence that is complementary to the nucleotide sequence of at least a portion of the target gene.

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16. (Amended) The method of claim 1 wherein the double stranded RNA comprises a nucleotide sequence that is complementary to a region of at least 50 bases of the target gene.

17. (Amended) The method of claim 1 wherein the double stranded RNA is supplied in an amount sufficient to completely inhibit expression of the target gene.

18. (Amended) The method of claim 1 in which the double stranded RNA comprises a single strand which is self-complementary.

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22. (Amended) The method of claim 20 wherein the embryo is supplied with the double stranded RNA using microinjection.

24. (Amended) The method of claim 23 wherein the cell is present in an organism, and the cell is supplied with the double stranded RNA by introducing double stranded RNA into a body cavity or interstitial space of the organism.

25. (Amended) The method of claim 23 wherein the cell is present in an organism, and wherein the cell is supplied with the double stranded RNA by delivering double stranded RNA to the organism via oral, topical, parenteral, vaginal, rectal, intranasal, ophthalmic, or intraperitoneal administration.

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26. (Amended) The method of claim 23 wherein the cell is present in a cell culture or a tissue explant, and wherein the cell is supplied with the double stranded RNA by incubating the cell culture or tissue explant in a solution comprising the double stranded RNA.

27. (Amended) The method of claim 1 wherein supplying the double stranded RNA to the cell comprises delivering double-stranded RNA to the cell, and wherein the double stranded RNA is treated with RNAse prior to delivery to the cell.

28. (Amended) The method of claim 1 wherein supplying the double stranded RNA to the cell comprises delivering double stranded RNA to the cell, the method further comprising, prior to delivering the double stranded RNA to the cell, annealing two complementary single stranded RNAs to yield the double stranded RNA.

29. (Amended) The method of claim 28 wherein the single stranded RNAs are annealed in the presence of potassium chloride.

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31. (Amended) The method of claim 1 further comprising introducing into the cell a second double stranded RNA in an amount sufficient to attenuate expression of a second target gene, wherein one of the strands of the second double stranded RNA is capable of hybridizing to the second target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C .

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32. (Amended) The method of claim 1 comprising introducing into the cell multiple double stranded RNAs in an amount sufficient to attenuate expression of multiple target genes, wherein one strand of each double stranded RNA is capable of hybridizing to the corresponding target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C.

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39. (Amended) The method of claim 23 further comprising identifying a phenotypic change in the cell culture associated with attenuated expression of the target gene.

42. (Amended) The method of claim 66 wherein the donor and the recipient are the same.

43. (Amended) The method of claim 66 wherein the donor and the recipient are different.

44. (Amended) The method of claim 66 wherein the tissue is fetal tissue.

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45. (Amended) The method of claim 66 wherein the donor and recipient organisms are vertebrates.

46. (Amended) The method of claim 66 further comprising identifying a phenotypic change in the tissue associated with attenuated expression of the target gene.

47. (Amended) The method of claim 66 wherein expression of the target gene is completely inhibited.

48. (Amended) A method for attenuating the expression of a target gene in a vertebrate cell comprising:

annealing two complementary single stranded RNAs in the presence of potassium chloride to yield double stranded RNA;

contacting the double stranded RNA with RNAse to purify the double stranded RNA by removing single stranded RNA; and

introducing the purified double stranded RNA into the cell in an amount sufficient to specifically attenuate expression of the target gene;

wherein one of the strands of the double stranded RNA is capable of hybridizing to the target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C, and wherein the double stranded RNA is formed from single-stranded RNA that is purified in the absence of phenol or chloroform.

57. (Amended) The method of claim 66 wherein the transplant tissue is hepatocytes.

61. (Amended) A kit comprising reagents for attenuating the expression of a target gene in a cell, the kit comprising:

a DNA template comprising two different promoters selected from the group consisting of a T7 promoter, a T3 promoter and an SP6 promoter, each promoter operably linked to a nucleotide sequence, such that two complementary single stranded RNAs are capable of being transcribed from the DNA template;

a plurality of primers for amplification of the nucleotide sequence;

nucleotide triphosphates for forming RNA;

at least two RNA polymerases, each capable of binding to a promoter on the DNA template and causing transcription of the nucleotide sequence to which the promoter is operably linked;

a purification column for purifying single stranded RNA;

buffer for annealing single stranded RNAs to yield double stranded RNA; and

RNAse A or RNAse T for purifying double stranded RNA;

wherein one of the strands of the double stranded RNA is capable of hybridizing to the target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C .

62. (New) The method of claim 1 wherein one of the strands of the double stranded RNA is capable of hybridizing to the target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 70°C.

63. (New) A method for attenuating the expression of a target gene in a vertebrate cell comprising delivering a double stranded RNA to the cell in an amount sufficient to specifically attenuate expression of the target gene, wherein the double stranded RNA comprises a nucleotide sequence that is complementary to a region of at least 25 nucleotides of the target gene, and wherein the double stranded RNA is formed from single-stranded RNA that is purified in the absence of phenol or chloroform.

64. (New) A method for attenuating the expression of two or more target genes in a vertebrate cell, the method comprising supplying the cell with two or more double stranded RNAs in an amount sufficient to specifically attenuate expression of the target genes, wherein one strand of each double stranded RNA is capable of hybridizing to the corresponding target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C.

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65. (New) A method for attenuating the expression of two or more target genes in a vertebrate cell, the method comprising supplying the cell with two or more double stranded RNAs in an amount sufficient to specifically attenuate expression of the target genes, wherein one strand of each double stranded RNA comprises a nucleotide sequence that is complementary to a region of at least 25 nucleotides of a target gene.

66. (New) A method for reducing or preventing an immune response in a recipient organism to a transplant tissue obtained from a donor organism, the method comprising supplying the transplant tissue with a double stranded RNA *in vitro* prior to implanting the transplant tissue into the recipient organism, wherein the double stranded RNA attenuates the expression of a target gene in the transplant tissue that can elicit an immune response in a recipient, and wherein one of the strands of the double stranded RNA is capable of hybridizing to the target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C.

67. (New) A pharmaceutical composition for inhibiting the function of a target gene in a vertebrate cell, wherein the composition comprises a double stranded RNA in

an amount sufficient to specifically attenuate expression of the target gene, wherein the double stranded RNA comprises a nucleotide sequence that is complementary to a region of at least 25 nucleotides of the target gene, and wherein the composition does not comprise phenol or chloroform.

68. (New) The method of any one of claims 48, 63, 64, 65 or 66 wherein the cell is a mammalian cell.

69. (New) The method of any one of claims 1, 48, 63, 64, 65 or 66 wherein the cell is a human cell.

70. (New) The method of any one of claims 23, 48, 63, 64, 65 or 66 wherein the cell is present in a mammal.

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71. (New) The method of claim 70 wherein the cell is present in a human.

72. (New) The method of claim 63 or 65 wherein the target gene is associated with a disease.

73. (New) The method of claim 63 or 65 wherein the target gene is associated with a disease from a pathogen.

74. (New) The method of claim 1, 64, 65 or 66 wherein the double stranded RNA is supplied to the cell by delivering to the cell a DNA encoding the double stranded RNA.